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*Supporting Information*

# Structural requirements for anti-oxidant activity of calix[n]arenes and their associated anti-bacterial activity

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# Experimental details:

## Synthesis and characterization of calix[n]arenes

All chemicals were purchased from ACROS Organics or Sigma Aldrich and used without further purification. Solvents were of chemical grade and were used without any purification.

The starting materials, calix[n]arenes, were prepared by deterbutylation of *para*-tert-butylcalix[n]arenes, using the procedure described by Gutsche [1].

*Para*-sulphonato-calix[n]arenes SC4a, SC6a and SC8a have been synthesized according to the procedure described by Coleman *et al*. [2].

Calix[n]arene-O-propyl-3-sulphonates SC4b, SC6b and SC8b were synthesized by the reaction of these calix[n]arenes with propane-1,3-sultone described for tBu calix[6]arene by Shinkai *et al*. [3].

*Para*-sulphonato-calix[n]arene-O-propyl-3-sulphonates SC4c, SC6c and SC8c have been prepared following the procedure described by Hwang *et al.* [4] and adapting the procedure of Shinkai *et al.* [5]

Calix[4]arene dihydroxyphosphonic acid (C4diP) par the method of Markovsky and Kalchenko [6].

All the physical characteristics of the synthetized calixarenes correspond to the literature values.

Sulphated β–cyclodextrin (CD) has been purchased from Sigma-Aldrich.

## Charge distribution, geometry and cavity sizes in the observed molecules

The ionisation state of the molecules is totally unclear: SC4a can be a tetra or penta anion and both of the higher analogs can have some degree of deprotonation at the phenolic face. For the other molecules the b and c series have deprotonated sulphonates but for the O-sulphonates it is not clear if there is full deprotonation. The C4diP is normally present as a di-anion but other states have been observed.

For the calix-4-arenes, SC4a is a cone and the other two molecules have hairy projections of the O-alkyl-sulphonate groups. The calix-6-arene and calix-8-arene molecules can take a large number of conformations, the representation (Supplementary Scheme 1) should be treated as a simplified schematic. The sulphated CD is fixed as a rigid cone. C4diP has always been observed as a cone.

With regard to cavity sizes little can really be said, the calix-4-arene derivatives all have cavities capable of including alkyl chains and small aromatic molecules, as does the cyclodextrin, however the larger calix-arenes possess cavities of varying geometry that can shift to best accommodate potential guest species.

## Synthesis and characterization of macrocyclic molecules (calix[n]arene or cyclodextrin) capped silver nanoparticles

10 mL of $10^{-2}$ M $AgNO_3$ solution was added to 80 mL of deionized water. To this solution, 10 mL of $10^{-2}$ M of the macrocyclic molecule aqueous solutions were added as stabilizers with stirring for 30 min. And then, 44 mg of $NaBH_4$ was added to the solution. The colloidal silver suspensions were obtained after 5 minutes.

A large excess of a strong reducing agent such as NaBH4 (15 times more concentrated than silver ions concentration in our study) completely reduces in an instant nucleation silver ions Ag+ into solid nanoparticles Ag0 [7]. This has been confirmed by the characterization of uniform sized silver nanoparticles of 45-48 nm by Transmission Electronic Microscopy and Dynamic Light Scattering for all the systems studied.

The macrocyclic molecules capped silver nanoparticles were then characterized by UV-Visible Absorption assays using a 96 well titre visible spectrometer (BioTek Power Wave 340). The presence of stable silver nanoparticles has been characterized by a maximum absorbance around 400nm corresponding to the plasmonic absorption peak. Yields of the nanoparticles are quantitative.

## Cell toxicity

Pheochromocytoma (PC12) neuronal-like cells were purchased from the American Type Culture Collection, and grown in 75 $cm^2$ tissue culture flasks in RPMI 1640 supplemented with 10% (v/v) heat-inactivated horse serum, 5% (v/v) heat-inactivated foetal calf serum, 1% GlutaMAX, 1% sodium pyruvate, 1% non-essential amino acids, 50 μg $mL^{-1}$ penicillin and 50 μg $mL^{-1}$ streptomycin. Cell culture reagents were purchased from Invitrogen unless otherwise indicated. Cultures were maintained at 37 °C in a humidified incubator containing 95% air and 5% $CO_2$. Cells were pre-plated on poly-L-lysine coated clear 96-well plates at a density of $10^4$ cells per well, stressed with 100mM glutamate (Sigma) and treated with 1, 10 or 100 μg $mL^{-1}$ calix[n]arene or cyclodextrin preparations in media. Note that calix[n]arene and cyclodextrin preparations were reconstituted in sterile growth media and no indications of contamination were observed. Control cultures had no synthesised molecules added. Following treatment, the preparations were removed and cultures were washed 3 times with HBSS, and incubated with 1 μM calcein-AM (Invitrogen) at 37 °C, 5% $CO_2$ for 30 minutes. Images were taken at 2 locations in triplicate culture wells at each concentration, using a Nikon Inverted Fluorescence microscope.

## Intracellular ROS generation

One day prior to ROS determination, cells were pre-plated on poly-L-lysine (10 μg mL−1, Sigma) coated black clear-bottomed 96-well plates at a density of $10^4$ cells per well. Intracellular accumulation of ROS was determined by pre-incubating cells with 10 μM chloromethyl dichlorodihydro-fluorescein diacetate (CM-H2DCFDA) (Life Technologies), for 60 minutes at 37 °C, 5% $CO_2$ in media. The dye was removed and cells were stressed with 100 mM glutamate (Sigma) and treated with 1, 10 or 100 μg $mL^{-1}$ calix[n]arene or cyclodextrin preparations in media or control as above. Cells were incubated for a further 60 minutes at 37 °C, 5% $CO_2$ and washed 2 times with Hank's balanced salt solution (HBSS) (without phenol red) at room temperature and resuspended in HBSS containing 1% triton X-100 to solubilise cells. Cellular fluorescence was measured on an Enspire multimode plate reader using an excitation wavelength of 480 nm and emission wavelength of 530 nm (Software version 4.1) (Perkin Elmer, USA). Each treatment was added to 5 replicate wells/ experiment and experiments were conducted 2-3 times. Results for each experiment were expressed as mean ± S.E.M. arbitrary units of fluorescence intensity. Note that data were not adjusted for protein content in the wells, as there were no reductions in cell numbers following treatment with the calix[n]arene or cyclodextrin preparations at the tested concentrations.

## References and Note

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## Supporting figures:

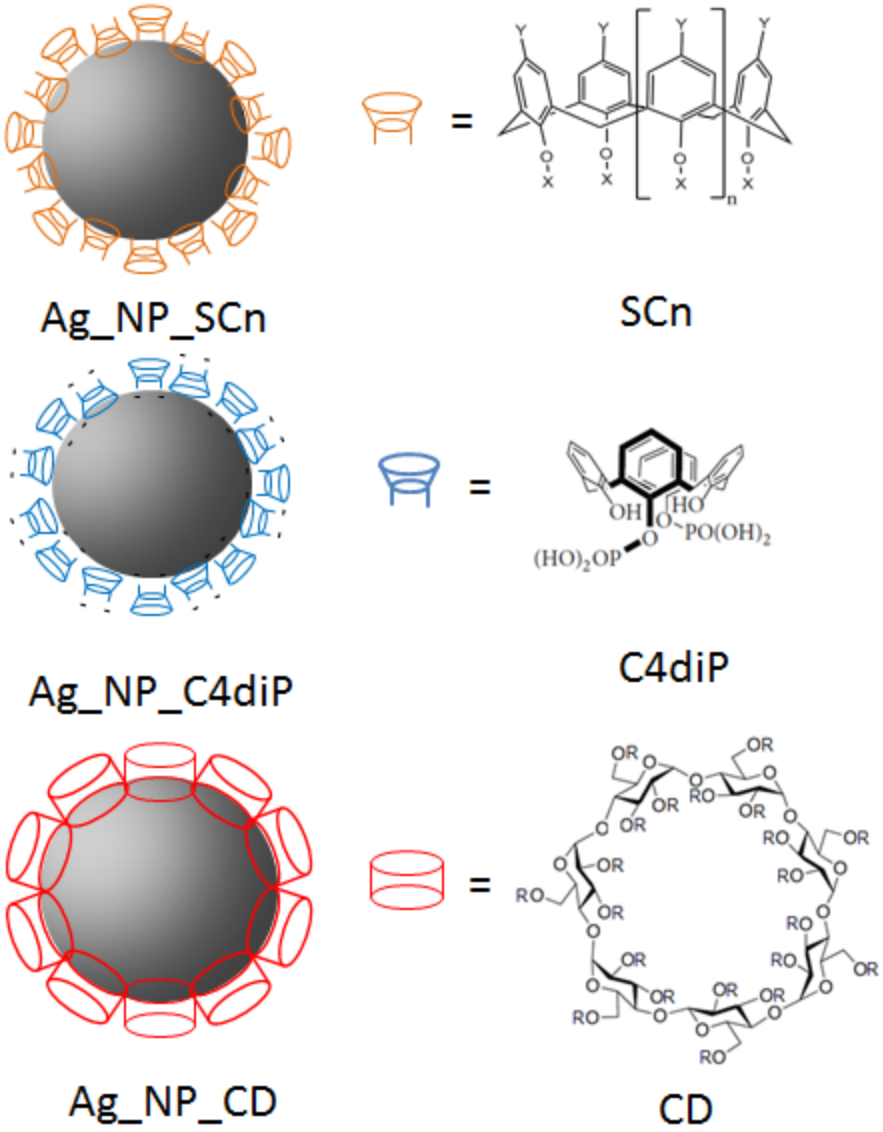


**Fig. S1.** Schematic representation of calix[n]arene and cyclodextrin capped on silver nanoparticles. SCn represents the general structure of sulphonato-calixarene derivatives with n=4, 6, 8; Y= H, $SO_3H$ and X= H, $-(CH_2)_3-SO_3H$. PC4 represents calix[4]arene dihydroxyphosphonic acid. CD represents sulphated β-cyclodextrin with R= $SO_3H$. At neutral pH, the sulphonates, sulphates and phosphonates groups of calix[n]arene and cyclodextrin capped on silver nanoparticles will be negatively charged.

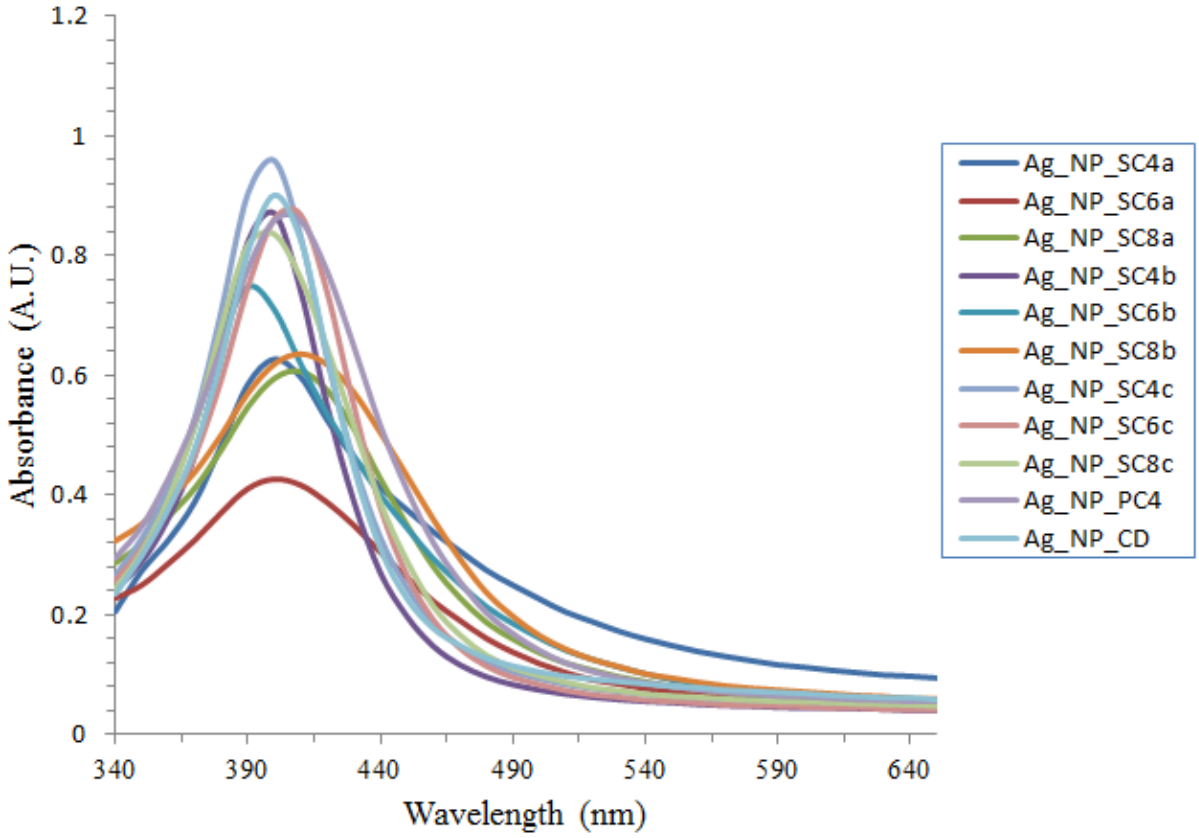


**Fig. S2** Characterization by UV-Visible spectrophotometry of the various macrocyclic molecules capped silver nanoparticles investigated here.